Variable temperature, relative humidity (0%–100%), and liquid neutron reflectometry sample cell suitable for polymeric and biomimetic materials

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We describe a variable temperature, relative humidity (0%–100% RH), and bulk liquid neutron reflectometry sample cell suitable for the study of polymeric and biomimetic materials (e.g., lipid bilayers). Compared to previous reflectometry cells, one of the advantages of the present sample environment is that it can accommodate ovens capable of handling either vapor or bulk liquid hydration media. Moreover, the design of the sample cell is such that temperature gradients are minimal over a large area (~ 80 cm^2) allowing for the nontrivial 100% RH condition to be attained. This permits the study, by neutron reflectometry, of samples that are intrinsically unstable in bulk water conditions, and is demonstrated by the lamellar repeat spacing of lipid bilayers at 100% RH being indistinguishable from those same bilayers hydrated in liquid water. © *2005 American Institute of Physics.* [DOI: 10.1063/1.1921550]

I. INTRODUCTION

In 1923, Compton reported on the total reflection of x rays from a solid sample with flat and smooth surfaces.[1] Over the last decade, x-ray and neutron reflectometry[2] have developed into popular and powerful techniques for the investigation of thin film phenomena. This popularity is primarily attributable to the significant development of experimental techniques,[3,4] instrumentation (e.g., synchrotron x-ray and cold neutron sources),[5] and theoretical/numerical techniques for analyzing experimental data.

The Fresnel equations describe the reflection and refraction of electromagnetic waves from the interfaces of dielectric media with different indices of refraction. In the case of thermal neutrons (e.g., $E \sim 0.02$ eV), the neutron index of refraction can be related to the scattering length density (SLD) of the material and Fresnel's equations apply equally well to the neutron wave function. The measured neutron specular reflectivity curve can thus be analyzed to determine the adsorbed film's total thickness, composition, periodicity, and even roughness.

For neutrons, reflectometry can typically probe film thicknesses ranging from 10 to 2000 Å, and is the technique of choice for many experiments examining surface effects in soft materials research, thin magnetic films, and multilayers. In a typical specular reflectivity experiment, a well-collimated monochromatic neutron beam passes through a single crystal of silicon (Si) and reflects from the solid–liquid or solid–air interface (Fig. 1). The resultant reflectivity curve can be directly related to the neutron SLD profile with a depth resolution, along a direction normal to the flat substrate surface (e.g., Si), of about 1 Å, whereas more detailed features of the profile can be determined with a spatial resolution of several Å.

An advantage in using neutron reflectometry for the study of soft materials rich in hydrogen is that neutrons, unlike x rays, are not only sensitive to light elements (e.g., H, C, N, O, etc.), but can also distinguish isotopic differences in these elements.[6] More importantly, the simple substitution of deuterium for hydrogen can substantially alter the SLD profiles of these hydrogen-laden films, while having a minimal effect on their chemistry.[7] By carrying out a series of reflectivity measurements on the same system, but with different deuterium labeled molecular components, a more accurate structure can be obtained. Recently, a phase-sensitive neutron reflectometry technique employing a buried reference layer has been developed, allowing for the direct inversion of reflectivity data to obtain unique compositional depth profiles of the films.[8] This is in lieu of conventional iterative fitting procedures which arrive at nonunique solutions.

Sample environments are a crucial aspect of any experimental setup. For example, "biologically relevant" conditions are understood by many as the following: (a) Lipids are in the liquid-crystalline L_α phase whereby no long-range order within the two-dimensional lipid bilayers exists as a re-

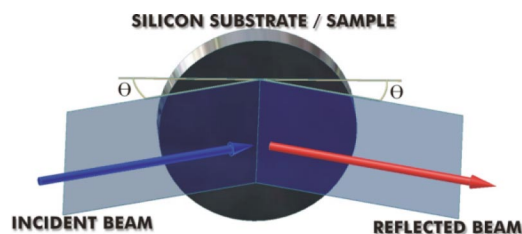[a] Author to whom correspondence should be addressed; electronic mail: john.katsaras@nrc.cnrc.gc.ca

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FIG. 1. (Color online). Reflection geometry showing the incident monochromatic neutron beam impinging on a silicon substrate at an angle Θ and subsequently reflected at an equal angle Θ. The angle between the straight through beam and the reflected beam is 2Θ.

sult of the rapid translational diffusion and *trans–gauche* isomerizations of the fatty acid chains. (b) Membranes are in so-called "excess water" conditions such that they can freely take up or give up water. (c) The aqueous environment reflects relevant physiological pH and ionic strength conditions. However, in certain cases where bulk water is in direct contact with biomimetic and other soft materials, sample degradation, or instability is a real concern.

Traditionally, reflectivity measurements of soft materials, particularly biologically relevant samples, have been carried out in a fashion whereby the hydrating medium, generally bulk water, is in direct contact with the sample. However, under these conditions some macromolecular assemblies can chemically degrade, or more commonly, become unstable (e.g., desorb from the substrate), making them inaccessible to experimentation lasting hours, or even days.[9] Since the chemical potential of water vapor in equilibrium with bulk water [100% relative humidity (RH)] is thermodynamically the same as that of liquid water, a way to overcome this limitation of sample instability is to hydrate the specimen in a 100% RH environment.[10] However, for decades samples hydrated in nominally 100% RH environments exhibited lamellar repeat spacings, d spacings, smaller than their counterparts hydrated in bulk water. Over the years, this discrepancy between these two methods of hydration came to be known as the "vapor pressure paradox," and was only resolved in 1998 when Katsaras, using thermal neutrons and a newly developed sample cell, demonstrated that the commonly accepted vapor pressure paradox was the result of inadequate sample environments containing substantial temperature gradients.[11] Subsequently, a variable humidity and temperature sample environment suitable for x-ray diffraction was constructed.[12,13]

These previous 100% RH cells, however, are only suitable for diffraction experiments, where the sample contains many hundreds of bilayers, resulting in intense Bragg reflections. On the other hand, in reflectometry the signal is not proportional to the sample volume but to the sample area interrogated by the neutron beam. Therefore, in order to maximize the signal we designed a sample cell capable of accommodating large Si substrates ($\sim$10 cm diameter), an area approximately 3–10 greater than the previous 100% RH environments.[11–13]

In biological systems, osmotic stress has been used to measure hydration forces between lipid bilayers, the forces and energies that control the assembly and the conformation

of molecules, membrane channel gating, and small molecule binding and enzyme function.[14,15] It is therefore evident, at least from a biological perspective, that the ability of a sample cell to accurately control RH is of great utility to certain types of science.

Here we report on a variable temperature (-20–$100\ ^\circ$C) neutron reflectometry sample cell capable of accurately controlling, through saturated salt solutions, RH. Compared to previous reflectometry cells,[16,17] one of the advantages of the present sample environment is that it can be adapted to either vapour or liquid hydration mediums. Moreover, the design of the cell is such that temperature gradients are minimal allowing for 100% RH to be attained over the large sample areas ($\sim$80 cm^2) required by neutron reflectometry studies. The sample cell is suitable for the study of a variety of materials, including polymeric and biomimetic materials.

II. CONSTRUCTION DETAILS

A neutron reflectometry sample is fabricated by adsorbing a thin film material on to a 10 cm diam Si crystal substrate. The sample/substrate is contained in a two-piece "oven" (Fig. 2), made entirely out of high purity aluminum,[18] capable of creating the requisite humidity conditions. The substrate fits snugly in a machined can with an o-ring that directly couples to a lid. Integral to the lid are three spring-loaded pins and a reservoir (Figs. 2 and 3) to hold saturated salt solutions. When assembled, the o-ring is compressed sufficiently against the lid to achieve a leak tight seal. The "face" of the Si crystal, the side where the sample is adsorbed, makes contact with the three spring-loaded pins which push the crystal firmly into the aluminum can with a 1 N force, eliminating any potential movement. This design not only allows for the unobstructed entry and exit of the neutron beam through either the air gap or the Si substrate, but also permits the use of varying thickness substrates. Two aluminum ovens were machined with 0.1 and 1 cm gaps between the faces of the 0.5 cm thick Si crystal and the lid.

The aluminum lid's liquid reservoir can contain saturated salt solutions for fixed humidity, or can accommodate a hydrating sponge for achieving 100% RH. In the latter case, a thin slice of porous sponge is held flat against the lid by a wire mesh which is fastened to the lid by several screws (Figs. 2 and 3). The sponge covers the entire surface area of the substrate allowing for a $\sim$0.5 cm air gap between the substrate and sponge face when in use with the 10 mm air gap oven. The sponge also has a "flap" that dips into the reservoir containing pure water to keep it constantly moist. It is this large evaporative surface area in close proximity to the substrate that maintains 100% RH across the entire sample. Without this feature, 100% RH conditions are nearly impossible to attain, as temperature gradients cannot be completely eliminated.[11]

The oven lid also contains two access ports, which can be used to add or remove liquid from the reservoir, or purge the air gap with a desired gas. The ports can be sealed during data collection.

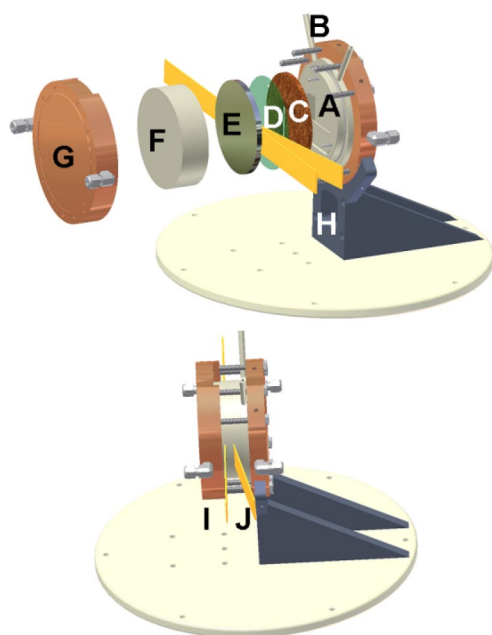Another key feature of the present 100% RH sample

FIG. 2. (Color online). Exploded view of the variable temperature 0%–100% RH neutron reflectometry sample cell (top), and the assembled view (bottom). Aluminum lid with an integrated liquid reservoir capable of accepting the various saturated salt solutions (A). The reservoir can be filled through one of the two access ports (B). Porous sponge (C) necessary in achieving 100% RH conditions and the stainless steel mesh (D) used to attach the porous sponge (C) to the aluminum lid (A). The silicon single crystal substrate (E) resides in an aluminum sample can (F), containing an o-ring, making a vapor proof seal with the aluminum lid (A). One of two massive liquid cooled/heated copper reservoirs (G) containing 125 ml of temperature regulated fluid. The two copper blocks are connected in series to a temperature controlled recirculating water bath. The aluminum lid (A) has three spring-loaded pins to press the Si substrate (E) firmly into the aluminum can (F). The pins are the only points of contact between the actual sample (e.g., lipid bilayers, polymer films, etc.) and the oven. A more detailed drawing of the pin assembly and oven lid is shown in Fig. 3. Support frame (H) machined from phenolic, grade X. (I) Incident monochromatic neutron beam. (J) Reflected neutron beam. The porous sponge is only required for 100% RH conditions.

cell, are the massive cooling/heating copper blocks, which also act as an integral structural feature (i.e., clamping device) of the assembled sample environment. The copper blocks each contain ∼125 ml of temperature regulated fluid that is continuously circulated between the blocks connected in series by a temperature controlled water bath. At the set temperature, the copper blocks, due to their mass, offer excellent temperature stability, ∼0.05 K over several hours. An exploded view of the components comprising the described RH sample cell is shown in Fig. 2.

The support frame for the sample oven/copper block assembly is machined from phenolic, grade X. Phenolic is a paper/resin composite that provides adequate strength for this application, and with a thermal conductivity coefficient of 7.0 cal/cm s °C makes for a good thermal insulator.

A separate sample cell was constructed for use with bulk liquids (Fig. 4). In this case, the oven consists of a machined, chemically inert poly-tetrafluoroethylene (PTFE) can with an o-ring that makes a seal when compressed against the face of the Si substrate. In this case, the Si substrate is an integral
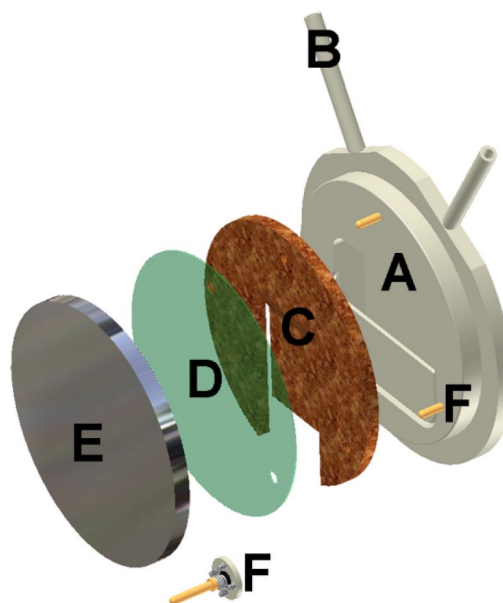

FIG. 3. (Color online). The lid portion of the aluminum sample oven shown in Fig. 2. The reservoir (A), designed to accept saturated salt solutions, may be filled through one of two access ports (B). The porous sponge (C) and stainless steel mesh (D) are integral components of the sample oven and are required for the attainment of 100% RH conditions. The Si substrate (E) is pushed firmly in place by three spring-loaded pins (F), the only points of contact with the sample that is adsorbed to the Si substrate. A detailed schematic of a single spring-loaded pin assembly is shown.

part of the bulk liquid oven. The gap between the face of the sample and the recessed portion of the PTFE contains ∼20 ml of liquid. The PTFE/substrate assembly is held together inbetween the copper blocks. Aluminum spacers allow for different thickness Si substrates to be accommodated with the appropriate pressure applied to the o-ring. This cell creates an unobstructed path for the incident and reflected neutron beams through the Si. In this case, the incident neutron beam enters from the "back" of the Si substrate (Fig. 4), whereas in the case of the 100% RH cell the incident neutron beam typically impinges on the "front" of the Si substrate (Fig. 2). The PTFE also contains two access ports, which allow for the filling and emptying of liquid with a minimal disturbance to the sample.

III. RESULTS

To lower the relative humidity from 100% to ∼99.9%, the sample chamber need only contain a temperature gradient of 0.01 K.[14] However, even this seemingly insignificant change in RH can result in an ∼5 Å decrease in the lamellar repeat spacing of lipid multibilayers such as egg phosphatidylcholine.[14] Traditionally, this problem has been avoided by immersing lipid multibilayers, and other samples adsorbed to a solid support, in the appropriate solvent.[19]

Measuring RH is not trivial as values can change significantly with slight variations in temperature and without any change in water content. Even so, the best RH sensors, which are nonlinear devices with temperature dependencies, have intrinsic accuracies of only ±1%. A better method for know-
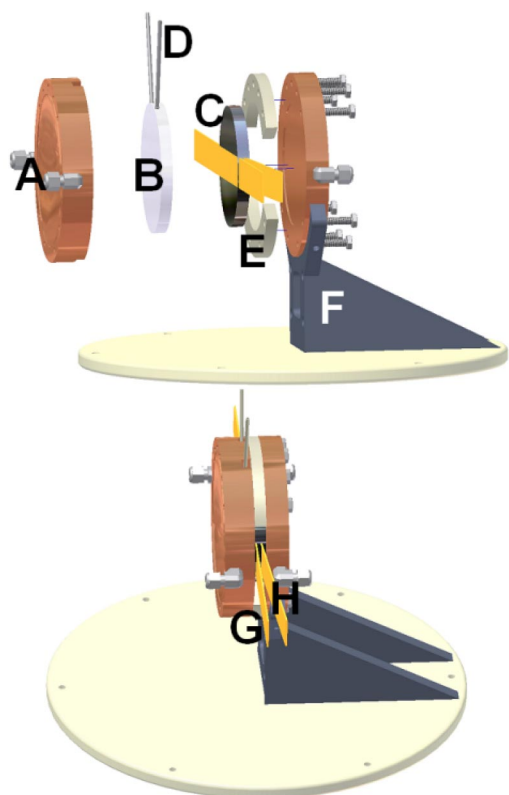
FIG. 4. (Color online). Exploded view of the variable temperature bulk fluid neutron reflectometry sample cell (top). Assembled view (bottom). One of two massive liquid heated/cooled copper reservoirs (A) containing 125 ml of temperature regulated fluid. The two copper blocks are connected in series to a temperature regulated recirculating water bath. The sample oven is made up of a PTFE portion containing the desired bulk liquid (B) and the silicon crystal substrate (C). The sample is adsorbed to the Si surface facing the bulk liquid, which is loaded into the PTFE reservoir using one of two access ports. Aluminum spacers and (E) phenolic grade X support base (F). The aluminum spacers allow for the incident neutron beam to directly impinge on the "back" side of the silicon, avoiding any interaction with either the cooling/heating copper blocks or the PTFE portion of the sample oven. Incident and reflected neutron beams, (G) and (H), respectively.
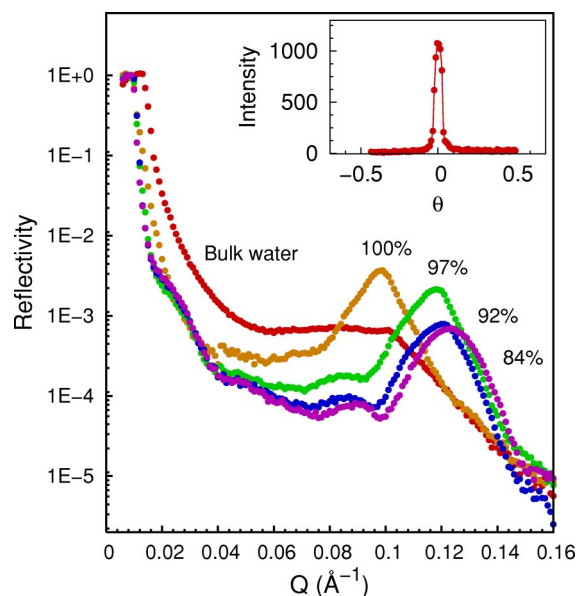

FIG. 5. (Color online). Reflectivity data using 2.37 Å neutrons and the sample cell shown in Fig. 2 of an aligned DMPC multibilayer stack consisting of five bilayers. The data sets are collected under specific RH conditions (84%–100%) at 306 K. It is evident that with increasing RH the quasi-Bragg peak moves to smaller Q values corresponding to increased values of d. The number of bilayers are estimated from the so-called Kiessig fringes, intensity oscillation occurring periodically along the reflectivity curve. The bulk water reflectivity data were obtained using the liquid sample cell shown in Fig. 4. The d-spacing values for 84%, 92%, 97%, and 100% RH are ∼50, 52, 53.2, and 64 Å, respectively. The error in d-spacing values is ±2 Å. The inset to the figure depicts the rocking curve for the sample at 100% RH condition and was obtained at a fixed 2Θ detector angle corresponding to $Q=0.098$, the position of the first order quasi-Bragg peak.

ing whether or not a sample cell has attained 100% RH is through the use of samples whose d spacings are well known.

Figure 5 shows reflectivity data from aligned dimyristoyl phosphatidylcholine (DMPC) multibilayers (five bilayers) at various RH (84%–100%) and 306 K. These data were collected using 2.37 Å neutrons and the sample cell shown in Fig. 2. From the reflectivity curves, it is obvious that with increasing RH the position of the broad quasi-Bragg reflection is continuously moving toward smaller values of Q ($2\pi/d$ spacing). This movement of the quasi-Bragg peak towards smaller Q values is indicative of DMPC bilayers swelling and moving further apart from each other. At 100% RH DMPC bilayers exhibit a d spacing of 64 ±2 Å, a value comparable to that from DMPC bilayers in bulk water.[11,19] Moreover, the number of DMPC bilayers is estimated from the so-called Kiessig fringes[20]—intensity oscillations occurring periodically along the reflectivity curve.[21]

The inset to Fig. 5 depicts a "rocking curve" at the d spacing of the 100% RH sample condition, an indication of how well the sample is aligned with respect to the Si sub-strate. Reflectometry data are collected in a Θ−2Θ scan mode, where the sample angle Θ is kept at half the detector angle 2Θ. By keeping the detector fixed at an angle corresponding to the maximum intensity of a Bragg reflection, in this case the first order quasi-Bragg peak corresponding to $Q=0.098$ Å^{-1}, the sample can be "rocked" around the specular reflection angle and the resultant signal is a measure of the amount of sample that is aligned to the specular reflected condition. The smaller the peak's full width at half maximum, the better the quality of the sample.

One reason for the construction of a 100% RH reflectometry sample cell is best exemplified by the bulk water reflectivity data shown in Fig. 5. When the same DMPC sample is placed in the PTFE bulk water reflectivity cell, it is evident that DMPC bilayers become unstable and possibly desorb from the substrate,[11,19] giving rise to poor quality data.

Figure 6 shows reflectivity data (top) from thin films composed of weak polyelectrolytes, poly(acrylic acid) and poly(allyl)amine hydrochloride (PAA/PAH). The polyelectrolyte multilayer films were assembled at solution pH 3.5, where PAA is partially charged, and PAH is fully charged. Bulk D_2O measurements show that the film thickness is homogeneous practically over the entire area (∼80 cm^2) of the Si substrate, as indicated by the periodic oscillations along the neutron reflectivity curve corresponding to the total sample thickness. These oscillations are Kiessig fringes, and are the result of the interference of waves reflected from the polymer/air and polymer/substrate interfaces.
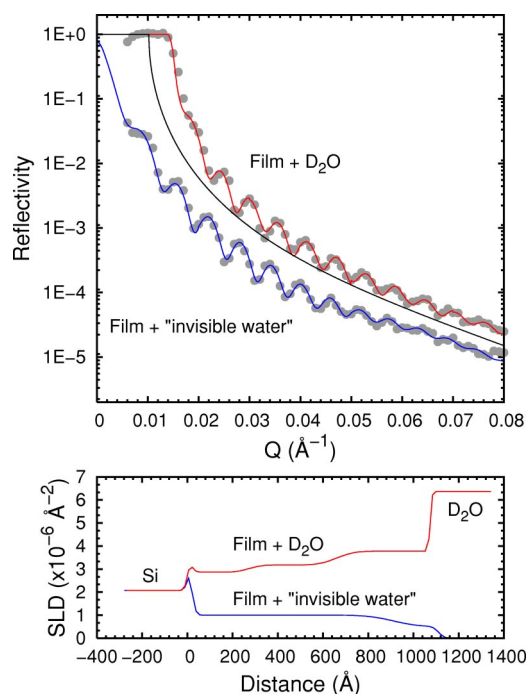
FIG. 6. (Color online). Neutron reflectivity data (top) of thin films composed of weak polyelectrolytes fabricated electrostatically onto a Si substrate in D_2O and invisible, or commonly referred-to, "contrast matched" water (92:8 H_2O:D_2O). One-dimensional SLD profiles (bottom) of poly-(acrylic acid) and poly(allyl)amine hydrochloride in pure D_2O and 92:8 H_2O:D_2O.

Using the SLD recursion formalism proposed by Parratt,[22] the reflectivity data are fit to a slab model resulting in the one-dimensional SLD profiles shown in the bottom panel of Fig. 6.[23] For the bulk D_2O measurements it is evident that D_2O predominantly sorbs near the polymer–ambient interface. Using a 92:8 mixture of H_2O to D_2O, a null scattering length is obtained, allowing for the deconvolution of contributions from the polymer film and absorbed water to the total scattering.

The sample cell described here has the single advantage, over previous reflectometry cells,[16,17] of achieving 100% RH. For example, due to the presence of substantial temperature gradients, the neutron reflectometry cell described in Ref. 17 was capable of attaining only 98% RH. That same reference also describes briefly a neutron sample cell used at the Institute Laue-Langevin (Grenoble, France), whereby 100% RH conditions were achieved by inducing a temperature differential between the water reservoir and the sample, the sample being slightly cooler. This induced temperature gradient method, in fact, does not achieve 100% RH conditions but causes the water saturated air to condense on the "cooler" sample, the obvious drawback being that over time the condensate "washes" the sample off of the substrate.

Another advantage of the present sample cell over the ones previously described[17] is that the large copper blocks eliminate the need for additional temperature stabilizing cans, which have to be designed in a manner as to provide "windows" with minimal neutron absorption. In our liquid cell, the neutron path is entirely through the sample. For the humidity cell, the neutron path is only through the walls of the highly transparent aluminum oven can, and the humid air gap inbetween, whose gaseous composition can be controlled, and therefore known.

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